REMARKS

Claim 1 has been amended to indicate that the cationic species have an additional proton, such that $(M+H)^+$ and that the anionic species have a proton taken away, such that $(M-H)^-$. Support for this amendment can be found at, for example, from page 43, line 20 through page 45, line 8 of the specification.

Claim 12 has been amended to indicate that the gel carrier or gel substance is polyacrylamide. Support for this amendment can be found at, for example, page 92, line 18.

Claim 1 has also been amended to improve its form.

Upon entry of these amendments, which are respectfully requested, claims 1-19 will be pending.

Starting on page 2 of the Office Action, claims 1-19 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Electrophoresis, 1998, Vol. 19, p.928-938 ("Ref. 1: Tsugita et al.") in view of Chemistry Letters, 1992, p.235-238 ("Ref. 2: Tsugita et al.") and U.S. Patent No. 5,952,653 ("Ref. 3: Covey et al."). Applicants presume the rejection is also based on as well (Polymer Bulletin, 1996) ("Ref. 4: Vogt et al."), because the Examiner's explanation of the § 103 rejection explicitly includes Vogt on page 7, fourth full paragraph.

Applicants respectfully submit that the presently claimed invention is not rendered obvious by the cited references for at least the following reasons.

As to Claims 1-11

Ref. 1: Tsugita et al. teaches such a process for C-terminal sequencing for the protein, which process comprises following steps:

The first step of extracting the protein from the protein spot on the polyacrylamide gel is carried out by using the following extraction procedure.

The protein spot was excised from the polyacrylamide gel and broken up by the use of a small hand-held homogenizer after addition to the 500 μL of 6M guanidine-HCl, 0.1% SDS, 0.5 M Bicine, 4mM EDTA, pH 8.0-8.5. The 6M guanidine-HCl and 0.1% SDS contained in the solution is successfully used to denature the protein, and thus the denatured protein can be easily extracted from the gel carrier to be collect in the pool of supernatant and two addition washes.

The denatured protein contained in the pooled supernatant is subjected to purification and separation with use of a mini-column of C18 silica. The denatured protein isolated by the column separation method is dried up to use as the dried protein sample.

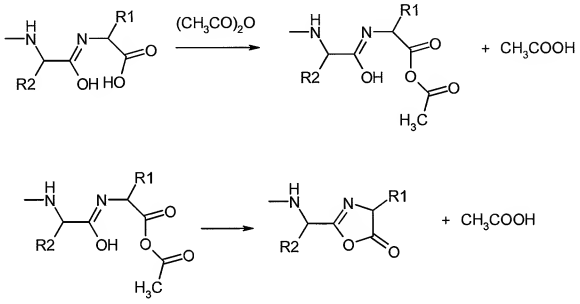
Therefore, Ref. 1: Tsugita et al. fails to teach any process for C-terminal sequencing, in which the reactions for C-terminal stepwise degradation are carried out for the peptide being maintained in a state that it is bound on the gel carrier, in particular, on the polyacrylamide gel.

Ref. 1: Tsugita et al. teaches such a procedure of reactions for C-terminal stepwise degradation used for the dried protein sample comprising the following three reaction sub-steps (i) – (iii):

(i) The first reaction sub-step for acetylation of the N-terminus of the peptide (denatured protein) and formation of an oxazolone at the C-terminal carboxyl group of the peptide (denatured protein):

Acetic anhydride with 20% acetic acid tetrahydrofuran solution in the present of 1% DTT was reacted on the dried sample of peptide (denatured protein) at 60 °C for 10 min. The reaction of formation of the oxazolone may be carried out by the following reaction scheme:

Formation of the oxazolone at the C-terminal carboxyl group:



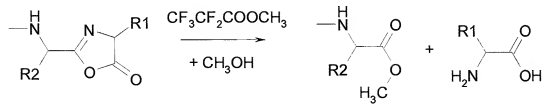
The acetic acid may be used as a catalyst for inducing the convention of keto-form of the amido moiety into the enol-form.

(ii) The second reaction sub-step for degradation of the oxazolone-ring to liberate the C-terminal amino acid and to form the esterified peptide:

The reaction is made with 5% PFPMe (pentafluoropropionic methyl ester: CF_3CF_2-CO-OCH_3) in methanol (CH_3OH) at 5 °C for 15 min.

The reaction of degradation of the oxazolone may be carried out by the following reaction scheme:

Degradation of the oxazolone:



The reaction mechanism may be alcoholysis in help of catalytic function of PFPMe (pentafluoropropionic methyl ester: CF_3CF_2-CO-OCH_3. The C-terminal amino acid was liberated to be dissolved in the methanol solution, and thus, the peptidyl reaction product was formed in the shape of esterified peptide.

Therefore, PFPMe (pentafluoropropionic methyl ester: CF_3CF_2-CO-OCH_3) was used as a catalytic agent for inducing the solvolysis reaction with use of methanol (CH_3OH) on the oxazolone-ring.

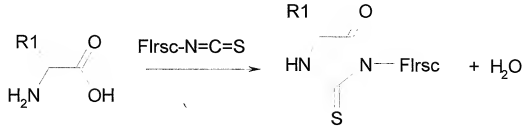
In view of this fact, Ref. 1: Tsugita et al. fails to suggest any reaction for degradation of the oxazolone-ring which would be achieved without methanol (CH_3OH).

Furthermore, Ref. 1: Tsugita et al. fails to provide any evidence suggesting that PFPA (pentafluoropropionic acid: CF_3CF_2-COOH) without methanol (CH_3OH) would be used as a reactant for the degradation of the oxazolone, in place of PFPMe (pentafluoropropionic methyl ester: CF_3CF_2-CO-OCH_3) with methanol (CH_3OH). Methanol (CH_3OH) is a well-known protic solvent.

Therefore, Ref. 1: Tsugita et al. fails to provide any evidence suggesting that PFPA (pentafluoropropionic acid: CF_3CF_2-COOH) without any protic solvent such as methanol (CH_3OH) would be used as a reactant for the degradation of the oxazolone-ring.

The C-terminal amino acid isolated in the form of free amino acid was modified with fluorescien isothiocyanate, and then analyzed by HPLC. The reaction scheme for the modification with fluorescein isothiocyanate may be shown as follows.

Modification with fluorescien isothiocyanate (Flrsc-N=C=S):



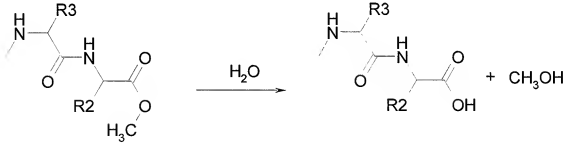
On the other hand, the esterified peptide collected from the reaction solution was subjected to the final reaction.

(iii) The final reaction sub-step for conversion of the esterified peptide (peptidyl methyl ester) into the peptide with a free carboxyl group at its C-terminus:

10 % DMAE aqueous solution was used at 60 °C for 20 min in the hydrolysis reaction of the ester bond to convert the esterified peptide (peptidyl methyl ester) into the peptide with a free carboxyl group.

The reaction of hydrolysis of ester may be carried out by the following reaction scheme:

Hydrolysis of ester:



The peptide with a free carboxyl group was collected from the aqueous solution, and then was dried up to use as a dried peptide sample for the next degradation step.

Accordingly, the peptidyl reaction product (peptide with a free carboxyl group), which is obtained in each of the C-terminal degradation steps, is by no means analyzed by mass spectroscopy.

Indeed, the C-terminal sequence of the denatured protein was made based on the HPLC analysis of the C-terminal amino acid (N-(pentafluoropropanoyl) amino acidic methyl ester) obtained in the sub-step (ii).

Therefore, the dried peptide sample, which has the C-terminal amino acid of –NH-CH(R2)-COOH to be analyzed in the next step of the C-terminal degradation, should be free from such contamination of the denatured protein that retains un-reacted C-terminal amino acid of –NH-CH(R1)-COOH.

At least, Ref. 1: Tsugita et al. fails to teach any process for preparation of such a mixture comprising the denatured protein and the series of the peptidyl reaction products (peptide with a free carboxyl group), each of which products has a step-wisely decreased amino acid sequence. Ref. 1: Tsugita et al. by no means uses FAB-MS or MALDI-TOF-MS for the process disclosed in 2.13 C-terminal sequencing.

Further, Ref. 1: Tsugita et al. also teaches such another process for multi-point C-terminal sequencing (i.e. Chemical specific cleavage and multiple C-terminal sequencing) for the protein, which process is carried out on the dried protein as well as on the polyacrylamide gel.

The process for the multi-point C-terminal sequencing for the protein sample on the polyacrylamide gel comprises the following steps (a) - (c):

(a) Step of electro-blotting the protein on the polyacrylamide gel to the Immobilon-CD membrane:

At first, the proteins were subjected to one-dimensional or two dimensional electrophoresis on the polyacrylamide gel. The resulted protein spots on the polyacrylamide gel were electroblotted to the Immobilon-CD membrane and negatively strained. The protein spot identified on the Immobilon-CD membrane was excised and cut into a 1 mm square.

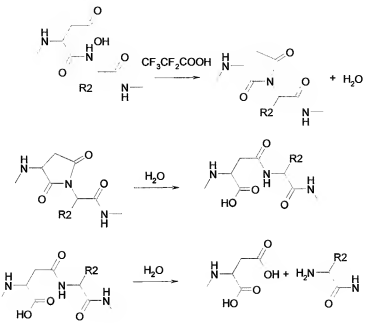
(b) Step of chemical specific cleavage of protein on the blotted membrane :

The cut-off square peace of the blotted membrane was put in the small tube and subjected to the specified cleavage reactions. In the specified cleavage reactions, the protein sample was cleaved at the carboxyl side of the aspartyl peptide bond (Asp-C), or at the amino side of the serine or threonine (Ser/Thr-N) peptide bonds, under the specified cleavage conditions, respectively.

Ref. 1: Tsugita et al. employed such a specified cleavage condition for the Asp-C cleavage reaction that a vapor phase reaction was made with a vapor generated from a 0.2 % PFPA aqueous solution containing 1 % w/v DTT at 90 °C for 4-16 h. The vapor generated from the 0.2 % PFPA aqueous solution containing 1 % w/v DTT at 90 °C may contain a vapor of PFPA, a vapor of H_2O and a vapor of DTT.

The group of peptidyl reaction products (peptide fragments) produced in the reaction of cleavage at the carboxyl side of the aspartyl peptide bond (Asp-C) will consist of the N-terminal peptide fragment having a newly exposed C-terminal aspartic acid, inner peptide fragments having a newly exposed C-terminal aspartic acid and the C-terminal peptide fragment.

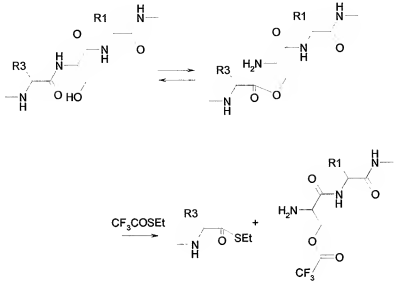
The Asp-C cleavage reaction may be made through the following reaction scheme.



Ref. 1: Tsugita et al. employed such a specified cleavage condition for the See/Thr-N cleavage reaction that a vapor phase reaction was made with a vapor of TFASEt (S-Ethyl trifluorothioacetate; $CF_3CO\text{-}S\text{-}CH_2CH_3$) at 30 °C for 24 h or at 50 °C for 6-24 h.

The group of peptidyl reaction products (peptide fragments) produced in the reaction of cleavage at the amino side of the serine or threonine (Ser/Thr-N) peptide bonds will consist of the N-terminal peptide fragment, inner peptide fragments having a newly exposed N-terminal Ser/Thr residue and the C-terminal peptide fragment having a newly exposed N-terminal Ser/Thr residue.

The Ser/Thr-N cleavage reaction may be made through the following reaction scheme.



(c) Step of extraction of the peptidyl reaction products from the cut-off square peace of the membrane.

After the specified cleavage reaction, the peptidyl reaction products (peptide fragments) were extracted with 30% and 60% acetonitrile aqueous solutions. The extract was dried and analyzed by FAB-MS or MALDI-TOF-MS.

However, Ref. 1: Tsugita et al. fails to teach any process in which chemical specific cleavage was carried out on the protein being maintained in a state that it is bound on the polyacrylamide gel in place of the protein on the blotted membrane.

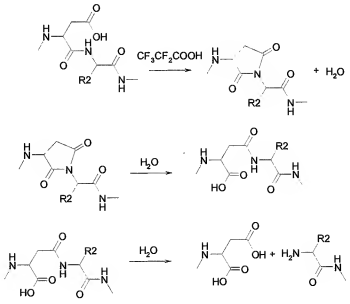
In view of these facts, Ref. 1: Tsugita et al. fails to provide any suggestion as to such a process for C-terminal stepwise degradation or for chemical specific cleavage, which is carried out on the peptide (denatured protein) being maintained in a state that it is bound on the gel carrier such as polyacrylamide gel.

Ref. 1: Tsugita et al. fails to provide any suggestion as to such a process for C-terminal stepwise degradation, which is suitable used for preparation of such a mixture comprising the

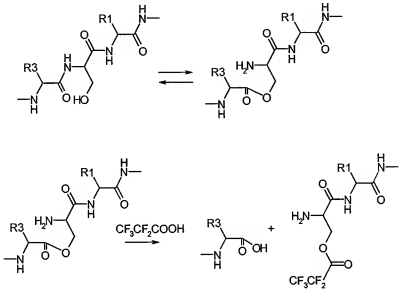
denatured protein and the series of the peptidyl reaction products (peptide with a free carboxyl group), each of which products has a step-wisely decreased amino acid sequence.

In addition, Ref. 1: Tsugita et al. also employed such a specified cleavage condition for the Asp-C cleavage reaction and Ser/Thr-N cleavage reaction and simultaneous successive C-terminal truncation reaction that these vapor phase reactions were made with a vapor of 90% PFPA (CF_3 CF_2COOH) aqueous solution containing 1% DTT at 90 °C for 1-16h. The vapor generated from the 90 % PFPA aqueous solution containing 1 % w/v DTT at 90 °C may contain a vapor of PFPA, a vapor of H_2O and a vapor of DTT.

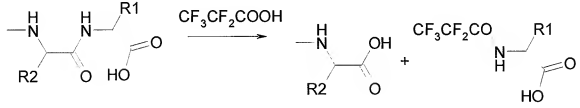
The Asp-C cleavage reaction may be made by using the vapor generated from the 90 % PFPA aqueous solution through the following reaction scheme.



The Ser/Thr-N cleavage reaction may be made by using the vapor generated from the 90 % PFPA aqueous solution through the following reaction scheme.

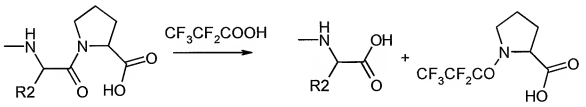


The simultaneous successive C-terminal truncation reaction may be made by using the vapor generated from the 90 % PFPA aqueous solution at 90 °C through the following reaction scheme.



Indeed, such a peptide of TQAGRDSFRESLSAL is formed from the peptide of TQAGRDSFRESLSALP by removing Pro in the simultaneous successive C-terminal truncation reaction (See Table 3). The C-terminal Pro residue can never be converted into the oxazolone-ring form.

Therefore, the C-terminal Pro residue may be removed by using the vapor generated from the 90 % PFPA aqueous solution at 90 °C through the following reaction scheme.



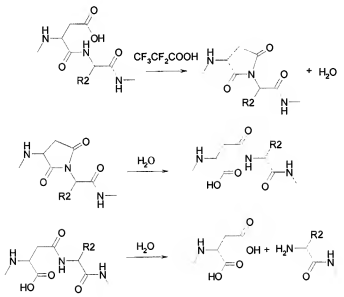
Further, Ref. 1: Tsugita et al. fails to provide any suggestion as to whether or not PFPA without any protic solvent, which is suitably used in the vapor phase reaction for the Asp-C cleavage reaction, would be employed as a reactant for the liquid phase reaction for degradation of the oxazolone-ring, in place of PFPMe with a protic solvent such as methanol (CH_3OH). At least, the function of PFPMe used in the liquid phase reaction for degradation of the oxazolone-ring is quite different from the catalytic function of PFPA used in the vapor phase reaction for the Asp-C cleavage reaction. Therefore, there is no good reason to believe that PFPA would have a similar function to that of PFPMe used in the liquid phase reaction for degradation of the oxazolone-ring.

Furthermore, Ref. 1: Tsugita et al. also teaches such another process for C-terminal sequencing at multiple sites for the protein, which process is carried out on the dried protein sample as well as the protein sample blotted on Immobilon-CD membrane. (See 3.4 C-terminal sequencing at multiple sites)

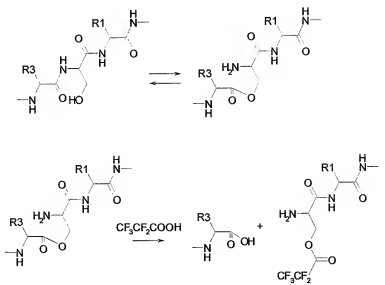
In the process for C-terminal sequencing at multiple sites, the dried protein sample or the protein sample blotted on Immobilon-CD membrane was reacted with the vapor of concentrated perfluoric acid, i.e. a vapor from a 90 % PFPA aqueous solution containing 1 % DTT at 90 °C for 2-16 h. The reaction with the aqueous vapor from 90 % PFPA aqueous solution at 90 °C for

2-16 h provided cleavage at the C-side of aspartic acid (Asp-C cleavage reaction) and cleavage the N-side of serine/threonine (Ser/Thr-N cleavage reaction), and simultaneous successive truncation at the C-termini of the cleaved fragments.

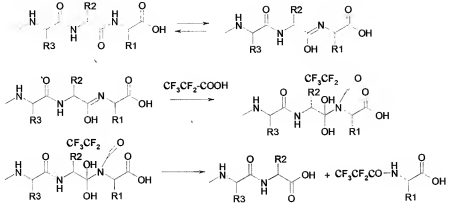
The Asp-C cleavage reaction may be made through the following reaction scheme.



The Ser/Thr-N cleavage reaction may be made through the following reaction scheme.



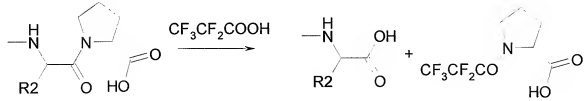
The reaction of the simultaneous successive truncation at the C-termini of the cleaved fragments may be made through the following reaction scheme.



In the reaction of truncation at the C-terminus of the cleaved fragment, the vapor of PFPA (CF_3CF_2COOH) may be used as a reagent.

Indeed, such a peptide of TQAGRDSFRESLSAL is formed from the peptide of TQAGRDSFRESLSALP by removing Pro in the simultaneous successive C-terminal truncation reaction (See Table 3). The C-terminal Pro residue can never be converted into the oxazolone-ring form.

Therefore, the C-terminal Pro residue may be removed by using the vapor generated from the 90 % PFPA aqueous solution at 90 °C through the following reaction scheme.



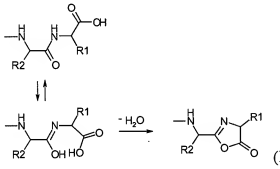
Therefore, the reaction scheme of the process used for C-terminal sequencing at multiple sites is concluded to be quite different from the reaction scheme of the process as claimed in Claims 1-11.

Accordingly, Ref. 1: Tsugita et al. fails to teach any process for preparing a mixture containing a series of peptidyl reaction products by chemically releasing the C-terminal amino acids successively, in which the oxazolone-ring is formed from the C-terminal amino acid, and any chemically cleavage of the peptide is successfully prevented in the chemical reaction step.

In contrast, the process for releasing the C-terminal amino acids successively from the peptide of the present invention is carried out through the following reaction schemes:

(I) reaction for formation of 5-oxazolone ring:

The reaction for formation of 5-oxazolone ring is expressed on the whole by the following reaction scheme (I):

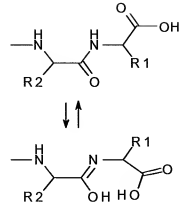


(I)

The reaction of scheme (I) consists of the following two stages (Ia) and (Ib).

(Ia) keto-enol tautomerism:

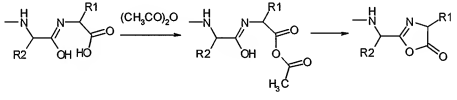
The perfluoroalkanoic acid contained in the mixed solution of the alkanoic acid anhydride and the perfluoroalkanoic acid dissolved in the dipolar aprotic solvent is allowed to act as a proton donor on the dried peptide at the stage of keto-enol tautomerism, as shown in the following reaction scheme (Ia):



(Ia)

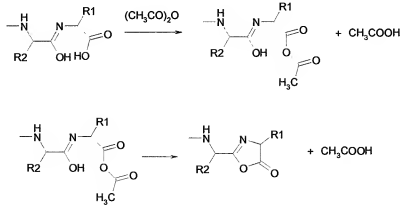
(Ib) formation of the activated C-terminal carboxyl group and formation of the intramolecular ester bond (formation of the 5-oxazolone ring):

The alkanoic acid anhydride is used as a reagent for formation of the activated C-terminal carboxyl group. The activated C-terminal carboxyl group is reacted with the hydroxyl group to form the 5-oxazolone ring.



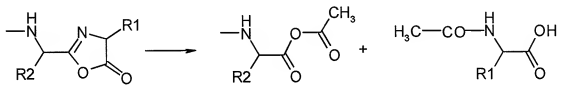
(Ib)

The following is a detailed reaction scheme of the stage (Ib):



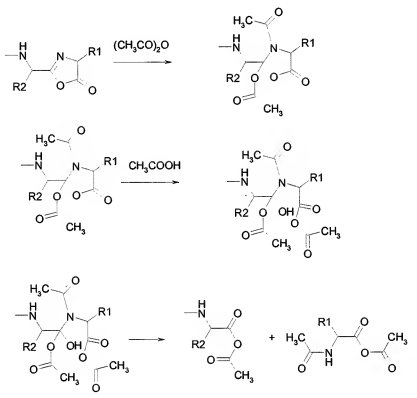
(II') separation of the C-terminal amino acid and formation of the reaction intermediate for the next stage:

The alkanoic acid anhydride is used as a reagent for the addition reaction on the double bond of >C=N- type of the 5-oxazolone ring. The degradation of the 5-oxazolone ring is made via such a reaction as shown by the following reaction scheme (II'):



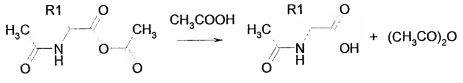
(II')

The following may be a detailed reaction scheme of the stage (II').



The alkanoic acid, which is a by-product from the alkanoic acid anhydride formed at the stage (Ia) is used as a reagent at the second reaction for opening of the ester bond therein.

In addition, the alkanoic acid also reacts on the derivative of the C-terminal amino acid having acid anhydride form, and thereby the C-terminal acid anhydride form thereof is converted into the C-terminal carboxyl group.



The peptidyl reaction product having the activated C-terminal carboxyl group of the third reaction is just ready for the formation of the 5-oxazolone ring at the next stage.

The considerable variation of the reaction speeds of those stages is successfully used to prepare a mixture comprising the original peptide and the series of peptidyl reaction products produced therefrom.

At least, the reaction schemes used in the process for releasing the C-terminal amino acids successively from the peptide of the present invention are quite different from those used in the process for C-terminal stepwise degradation or in the process for C-terminal sequencing at multiple sites disclosed in Ref. 1: Tsugita et al.

Ref. 2: Tsugita et al. 1992 teaches a reaction of pentafluoropropionic anhydride (PFPAA: $(CF_3CF_2\text{-}CO)_2O$) vapor on polypeptide, in which a vapor phase reaction is made with the pentafluoropropionic anhydride (PFPAA: $(CF_3CF_2\text{-}CO)_2O$) vapor at such a low temperature as -18 °C.

However, Ref. 2: Tsugita et al. 1992 fails to provide any suggestion as to the reaction condition used for the liquid phase reaction used in the process for C-terminal stepwise degradation disclosed in Ref. 1: Tsugita et al.

Further, Ref. 2: Tsugita et al. 1992 also suggested such a reaction scheme for the C-terminal successive degradation of polypeptides, in which the vapor of PFPAA ($(CF_3CF_2\text{-}CO)_2O$) from 10% PFPAA in acetonitrile (CH_3CN) is used as a reagent for the sub-step of formation of oxazolone form the C-terminal amino acid of the peptide, and the vapor of PFPA derived from PFPAA is used as a reagent for the sub-step of degradation of the oxazolone ring. The vapor of pentafluoropropionic acid ($CF_3CF_2\text{-}COOH$) is in site formed from pentafluoropropionic anhydride ($(CF_3CF_2\text{-}CO)_2O$) at the sub-step of formation of oxazolone.

In contrast, Document 1: US 5,521,097 A which is US counterpart of JP 05-133958 (A1) listed in IDS submitted on June 9, 2005 provides good evidence suggesting that the reaction scheme as shown in p. 236 of Ref. 2 is by no means reasonable.

The three of inventors of Document 1: US 5,521,097 A filed on August 18, 1992; i.e. Akita Tsugita, Keiji Takamoto, and Kazuo Satake are just the authors of Ref. 2: Tsugita et al. 1992.

EXAMPLE 9 of Document 1: US 5,521,097 A describes the C-terminal successive degradation reaction of the dodecapeptide of Ala-Arg-Gly-Ile-Lys-Gly-Ile-Arg-Gly-Phe-Ser-Gly, in which reaction vapors from 10 % anhydrides of PFPA in acetonitrile at -18 °C were applied for 2 hours to the dried dodecapeptide. The reaction condition used for the C-terminal successive degradation reaction of the dodecapeptide of Ala-Arg-Gly-Ile-Lys-Gly-Ile-Arg-Gly-Phe-Ser-Gly is very similar to that used in Ref. 2: Tsugita et al. 1992

Further, EXAMPLE 8 of Document 1: US 5,521,097 A describes the C-terminal successive degradation reaction of the octapeptide of Lys-Lys-Lys-His-Pro-Asp-Tyr-Ile, in which reaction vapors from 10 % anhydrides of TFA, PFPA and HFBA in acetonitrile at -18 °C were applied for 2 hours to the dried octapeptide. FIG. 16 of Document 1: US 5,521,097 A

shows observed resulting peptides 1-8, 1-7, 1-6, 1-5, 1-4, 1-3 and 1-2. The resulting peptide 1-4 is Lys-Lys-Lys-His, in which Pro residue of the peptide 1-5 of Lys-Lys-Lys-His-Pro is removed.

The C-terminal Pro residue can never be converted into the oxazolone-ring form.

Therefore, the C-terminal Pro residue may be removed by using the vapor generated from the 10 % PFPA solution in acetonitrile at -18 °C though a reaction scheme other than the reaction scheme as shown in p. 236 of Ref. 2. In view of this fact, EXAMPLE 8 of Document 1: US 5,521,097 A provides good evidence suggesting that the C-terminal successive degradation reaction of the octapeptide of Lys-Lys-Lys-His-Pro-Asp-Tyr-Ile may be achieved through a reaction scheme other than the reaction scheme as shown in p. 236 of Ref. 2.

In addition, the reaction scheme as shown in p. 236 of Ref. 2 suggests such a by-product having a moiety of $-NHCH_2R_{12}$ derived from its C-terminal amino acid. The by-product having a moiety of $-NHCH_2R_{12}$ derived from its C-terminal amino acid can never be converted into the oxazolone-ring form.

The reaction scheme as shown in p. 236 of Ref. 2 suggested that the amount of the by-product having a moiety of $-NHCH_2R_{12}$ should be relatively increased in comparison with the amount of original peptide as the reaction time is increased.

However, Fig. 1 of Ref. 2: Tsugita et al. 1992 fails to provide good evidence suggesting that the amount of the by-product having a moiety of $-NHCH_2R_{12}$ would be relatively increased in comparison with the amount of original peptide as the reaction time is increased

This fact also provides good evidence suggesting that the reaction scheme of C-terminal successive degradation as shown in p. 236 of Ref. 2 is very questionable.

In conclusion, in view of Document 1: US 5,521,097 A, Ref. 2: Tsugita et al. 1992 fails to provide any good experimental evidence suggesting that the reaction scheme of C-terminal

successive degradation as shown in p. 236 of Ref. 2 would be reasonable. At least, EXAMPLE 8 of Document 1: US 5,521,097 A and the result of the by-product having a moiety of –$NHCH_2R_{12}$ derived from its C-terminal amino acid as shown in Fig. 1 of Ref. 2: Tsugita et al. 1992 provide good evidence suggesting that the reaction scheme of C-terminal successive degradation as shown in p. 236 of Ref. 2 is very questionable.

Furthermore, in view of the suggested reaction scheme, Ref. 2: Tsugita et al. 1992 fails to provide such a suggestion that PFPA may be used as a catalytic agent inducing the degradation of the oxazolone ring rather than a reagent involved in the degradation of the oxazolone ring.

At least, Ref. 2: Tsugita et al. 1992 fails to provide such a suggestion that PFPA (CF_3CF_2-COOH) may be used as a catalytic agent inducing the degradation of the oxazolone ring by alcoholysis with methanol in place of PFPMe (CF_3CF_2-$COOCH_3$).

Ref. 3: Covey et al. teaches such a procedure of enzymatic digestion of the long peptide by trypsin to cleave the long peptide into tryptic fragments.

Ref. 3: Covey et al. also teaches such a double charge rule that the tryptic fragment having Arg or Lys at the C-terminus thereof will be doubly positively charged in such form of $(M+2H)^{2+}$ by using Ion spray process for Ion Evaporation Mass Spectrometry, but that there are three exceptions to the double charge rule as follows:

First exception: such a tryptic fragment having other amino acid than Arg or Lys at the C-terminus thereof will only be singly charged by using Ion spray process for Ion Evaporation Mass Spectrometry.

Second exception: such a tryptic fragment having an amino terminus which is carboxylated or blocked (e.g. N-acylation at the N-terminus) will only be singly charged by using Ion spray process for Ion Evaporation Mass Spectrometry.

Third exception: such a tryptic fragment having Arg or Lys at the C-terminus thereof and containing an internal His will be triply charged in small percentage, but will be doubly charged in most percentage.

However, Ref. 3: Covey et al. fails to provide any suggestion as to whether or not such a double charge rule will be also observed for MALDI-TOF-MS or FAB-MS.

At least, Ref. 3: Covey et al. fails to provide any suggestion as to intensity of a singly positive charged ion of $(M+H)^+$ from the tryptic fragment having Arg or Lys at the C-terminus thereof to be measured by MALDI-TOF-MS. Ref. 3: Covey et al. fails to provide any suggestion as to intensity of a singly negative charged ion of $(M-H)^-$ from the tryptic fragment having other amino acid than Arg or Lys at the C-terminus thereof to be measured by MALDI-TOF-MS.

At least, Ref. 3: Covey et al. fails to provide such a suggestion that a singly positive charged ion of $(M+H)^+$ from the tryptic fragment having Arg or Lys at the C-terminus thereof may show stronger intensity in the spectrum of the cationic species of $(M+H)^+$ measured by MALDI-TOF-MS.

As to Claims 12-19

At first, Ref. 1: Tsugita et al. fails to teach any process in which chemical specific cleavage was carried out on the protein being maintained in a state that it is bound on the polyacrylamide gel in place of the protein on the blotted membrane.

Accordingly, Ref. 1: Tsugita et al. fails to provide any suggestion as to such a process for C-terminal stepwise degradation or for chemical specific cleavage, which is carried out on the

peptide (denatured protein) being maintained in a state that it is bound on the gel carrier, in particular on the polyacrylamide gel.

The process as claimed in Claims 12-19 employs a liquid phase reaction in place of a vapor phase reaction.

At least, the reaction schemes used in the process for releasing the C-terminal amino acids successively from the peptide are quite different from those used in the process disclosed in Ref. 1: Tsugita et al.

Ref. 2: Tsugita et al. 1992 teaches a reaction of pentafluoropropionic anhydride ($(CF_3CF_2\text{-}CO)_2O$) vapor on polypeptide, in which a vapor phase reaction is made with the pentafluoropropionic anhydride ($(CF_3CF_2\text{-}CO)_2O$) vapor at such a low temperature as -18 °C.

However, Ref. 2: Tsugita et al. 1992 fails to provide any suggestion as to the reaction condition used for the liquid phase reaction in place of the reaction condition used for the vapor phase reaction in the process for C-terminal stepwise degradation disclosed in Ref. 1: Tsugita et al.

Ref. 4: Vogt et al. teaches such a process for preparation of a high reactive gel-suspention of carboxymethyl cellulose (CMC), in which the polymer (carboxymethyl cellulose) is treated in a dipolar-aprotic solvent, such as N,N-dimethylacetamide and dimethylsulfoxide (DMSO), with p-toluene-sulfonic acid.

Ref. 4: Vogt et al. also provides such assumed mechanism that the activation (swelling in the dipolar-aprotic solvent) is achieved via an interaction between the carboxylate groups ($\text{-}CH_2\text{-}COONa$) of Na-CMC and HO_3S-groups of the p-toluene-sulfonic acid with a rapid exchange of the acidic hydrogen as well as an interaction of the lipophilic toluene unit of the p-toluene-sulfonic acid with the solvent.

Accordingly, Ref. 4: Vogt et al. fails to teach any process for preparation of gel-suspension of CMC in the dipolar-aprotic solvent without p-toluene-sulfonic acid.

Ref. 4: Vogt et al. also reported that an effective method for activation of CMC is precipitation of an aqueous solution of CMC by N,N-dimethylformamide (DMF) and the removal of the water from the swollen gel by repeated distribution under reduced pressure. This report may indicate that N,N-dimethylformamide (DMF) can never remove water from the water-swollen gel of CMC.

Further, Ref. 4: Vogt et al. reported that other acids like methane sulfonic acid, trifluoroacetic acid and monochloroacetic acid do not swell CMC to a comparable extent. Furthermore, Ref. 4: Vogt et al. reported that polysaccharides with directly at the polymer backbone bound carboxy groups like sodium alginate, sodium pectinate, and 6-carboxy cellulose do also not swell in the manner described for CMC.

In view of these facts, Ref. 4: Vogt et al. fails to provide any suggestion as to whether or not the combinational use of the dipolar-aprotic solvent with other acid than p-toluene-sulfonic acid is successfully applied for preparation of non-aqueous swelling gel of other polymer than CMC.

At least, Ref. 4: Vogt et al. fails to provide any suggestion as to whether or not the combinational use of the dipolar-aprotic solvent with perfluoroalkanoic acid is successfully applied for preparation of non-aqueous swelling gel of other polymer than CMC.

Further, Ref. 4: Vogt et al. fails to provide any suggestion as to whether or not the use of the dipolar-aprotic solvent without p-toluene-sulfonic acid is successfully applied for preparation of non-aqueous swelling gel of other polymer than CMC.

In particular, Ref. 4: Vogt et al. fails to provide any suggestion as to whether or not the use of the dipolar-aprotic solvent without p-toluene-sulfonic acid is successfully applied for preparation of non-aqueous swelling gel of polyacrylamide in quite similar manner to the case of CMC.

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

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